Title: Immunization with BCG vaccine starting after age 1 is associated with increased risk of IDDM in Quebec.

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I. Introduction.

Data from diabetes prone NOD mice indicates that immunization starting at birth is associated with a decreased risk of diabetes while immunization starting after 2 months can be associated with an increased risk of diabetes (1). Immunization starting at birth also helped prevent the development of diabetes in BB rats, development of lupus in MRL/lpr mice (1) and organ specific autoimmune disease in animal models (2). These studies were unique in that they used very low doses, doses based on humans doses but reduced proportionally for the weight of the rodents, of killed human vaccines such as a DTP vaccine and a anthrax vaccine. Others have shown immunization with BCG vaccine and other microbial agents can prevent diabetes in NOD mice (3). The timing of immunization was less important in these studies but the doses used were large, based on the relative size of mice compared to humans. Large doses of BCG may alter the immune system by causing chronic infections, anergy, or persistent immune stimulation. Others have also shown that certain immune stimulants can induce the onset of diabetes in rodents including poly I:C (4) and fecal material (5).

Epidemiology studies were performed to determine if the phenomenon we observed in NOD mice (1) could also be occurring in humans. Ecological data from Europe showed countries immunizing with BCG at birth had a lower risk on IDDM and countries immunizing starting at school age had a higher incidence of IDDM compared to countries not immunizing with BCG (6). Interestingly immunization at school age is not associated with such a large risk of IDDM if the vaccine is also given at birth since many of the countries which give BCG at birth and also give booster shots at school age have a lower risk of IDDM then unimmunized groups. An analysis of birth cohort data from Sweden (7) indicated that immunization at birth with BCG was associated with a decreased risk of IDDM. In both the European ecological study and the Swedish cohort study immunization with one dose of BCG was associated with the a decrease of about 50 cases of IDDM per 100,000 children immunized (6). Further analysis of the Swedish data indicated that the cumulative incidence of IDDM lines started to become almost parallel in the BCG and non BCG vaccinated cohorts around age 7 indicating the decreased risk of IDDM occurred largely in children 7 or under.

The European ecological data indicated immunization with BCG starting at school age may be associated an extra 100 or more cases of IDDM/ 100,000 children immunized. These extra cases of IDDM associated with BCG occured within 4 years of immunization since in the ecological studies most of the children were immunized at age 10 or greater and the incidence of IDDM was calculated in children under the age of 15. Based on the ecological data (6) immunization starting at school age compared to not immunizing was associated with an relative risk of 1.74 and immunization starting at school age compare to immunization at birth was associated with an relative risk of 2.6 in the 0-14 age group.

A case control study on the effect of BCG vaccination on IDDM was performed using a diabetes registry in Quebec (8). The initial analysis of the data was not specifically designed to test the findings from the ecological study on BCG immunization (6). We attempted to determine if the following hypotheses, based on the ecological data, is supported by the Quebec database. The first hypothesis is that immunization with BCG starting at birth is associated with a decreased

risk of IDDM in children under 7. The second hypothesis is that immunization with BCG starting after 2 months of life is associated with an increased risk of IDDM with the majority of these cases occurring within 4 years after immunization.

II. Methods

The Quebec IDDM and BCG registries have been described in full earlier (8). The diabetes registry included all new cases of IDDM occurring in children living in Quebec and diagnosed between January 1971 through December 1991. The BCG registry was started and maintained by a different group and the data was not specifically recorded for performing epidemiology studies. The information kept on BCG immunization listed whether the recipient received the vaccine in the first year of life but did not clarify if the vaccine was given at birth or some time after 2 months of life. It is assumed based on the prevailing immunization practices that the vast majority of children immunized in the first year of life were immunized at birth. The registry also failed to record about 15% of children that received the vaccine.

Data in the present study was tabulated to reanalyze the initial published studies described by Parent et al. (8). The published analysis involved two case control studies called Series A and Series B. Series A included 93 diabetics and 2903 controls age 7 through 18. The controls were collected as part of another study and the cases of IDDM from the diabetes registry were retrospectively chosen to match the controls in age, location of residency, and year of birth. Series B contained 249 cases of IDDM and 431 prospectively collected matched controls age 0 through 18.

We arranged Series A data to study the effect of the BCG vaccine when given starting after the first year of life ***(Table 1)****. The time of first dose of BCG was stratified by age at first immunization. Series B data was analyzed to study the effect of BCG immunization starting at birth and after year 1* ***(Table 2)****.*

Statistical analysis of the data was performed using a one-tail difference in population proportions as well as pearson's chi-square analysis and a one-tail Fisher's test of a 2x2 table. The stratified analysis combining data from both ***Tables 1*** *and* ***Tables 2*** *was performed using a Mantel-Haenszel weighted odds ratio.*

III. Results

1. Analysis of Series A

The analysis of Series A data ***(Tables 1 & 3)*** *shows that immunization with BCG starting after age 5 or age 7 is associated an increased risk of IDDM, odds ratio of 3.0.*

2. Analysis of Series B

Data from Series B ***(Tables 2 & 3)*** *shows immunization starting after the first year is associated with an increased risk of IDDM, odds ratio equal to 2.*

3. Combined Analysis of Series A and B.

A stratified analysis was performed combining information from Series A and Series B from the case control studies above. The combined analysis indicated immunization with BCG starting at least 1 year after birth was associated with an increased risk of IDDM, odds ratio of 2.32 (p=0.019), (95% confidence interval 1.17<2.31<4.80) in children 18 and under ***(Table 3).***

IV. Conclusion

There has been discrepancy in the published literature among different authors regarding the effect of vaccines on IDDM. A first glance the published results of the European ecological study (6), appeared to differ from the Quebec study (8). Authors from both papers worked together to try to explain the differences between the two papers. The joint analysis of the Quebec data however shows that the findings are consistent with the European ecological data and explains why there appeared to be initial differences.

The initial analysis of the Quebec data (8) showed that immunization with BCG vaccine was associated with an increased risk of IDDM, odds ratio of 1.26 in Series B. This calculation did not take into consideration whether the BCG vaccine was given at school age or birth and did not take into consideration the time between immunization and when the study was started. The data from Classen and Classen (6) indicated that immunization at birth may be associated with a decreased risk of IDDM while immunization starting at school age is associated with an increased risk of IDDM. Furthermore the data indicated that the majority of the effect of a BCG vaccine on IDDM may occur within 4 years or sooner when given at school age and by age 7 when given at birth.

In order to compare the Quebec data in light of the findings of Classen and Classen the data was analyzed according to the age at which BCG was received. In the model of Classen and Classen any children immunized starting after 2 months of life would have been considered at increased risk of IDDM if the children had been vaccinated at the time the study was in progress. However, Series A included only children over 7 years of age. We were concerned that cases of vaccine induced IDDM would not be detected in children who received the BCG vaccine between the ages of 1 and 5 because these cases of IDDM would occur before the study inclusion age of 7. To decrease the problem of under estimating cases of vaccine induced diabetes in children under 7 we included only those children immunized after the 5th birthday as being in ***the expected high risk group however we also analyzed the odds ratio in children immunized starting after their 7 birthday. In both cases the odds ratio was 3. This was consistent with the findings the results from series B where BCG immunization starting after age 1 was associated with an odds ratio of 2. The combined analysis indicated an odds ratio of 2.3.*** These findings are consistent with the ecological data (6) of children under 15 (6), which revealed immunization starting in school age associated with an odds ratio of between 1.7 and 2.6. ***By contrast we found that BCG immunization starting after age 1 in Series A was associated with an odds ratio of approximately 1.25. The later is consistent with the hypothesis vaccines given to children aged 1-3 caused diabetes in children before the diabetes inclusion age of 7.***

Our analysis revealed that the data was insufficient to determine if BCG vaccine administered at birth is associated with a decreased risk of IDDM. The immunization records

could not distinguish whether a child was immunized starting at birth or later in the first year. The later scenario would be expected to be associated with an increased risk of diabetes. Secondly data from Sweden indicated that a substantial proportion of cases of IDDM prevented by BCG may occur before age 7. However, the Quebec registry lacked the power to detect an effect before age 7 because of the absence of children under age of 7 studied in Series A and a lack of children under age 7 who were immunized at birth in Series B. Only 3.5% of the 170 children under the age of 7 in Series B , 2 diabetics and 4 controls, were immunized with BCG at birth.

We checked to see if confounding factors may explain the association between BCG immunization and IDDM however we were unable to find an explanation. We were most concerned about confounding variables affecting the analysis of Series A because this study contained retrospectively collected controls. Series B by contrast had prospectively matched controls and confounding variables were much less likely. We looked to see if the area of residence or year of birth could explain the association in Series A. We found that in Series A there were differences between the control and diabetic groups in regards to area of residency and year of birth however there were too few data points to analyze whether these factors could explain the association. As described above for Series A, of the 150 controls that were immunized starting after the first year of life, the age at immunization was not recorded in 27. We treated these conservatively as if they had been immunized after age of 5. If we had treated these controls as if they were immunized earlier than age 5 the odds ratio would increase from 3 to 7.7. In the analysis of children immunized after the 7th birthday the odds ratio would increase from 3 to 23. It is possible that those of British decent are more likely to receive BCG vaccination at school age then those of French decent and ethnicity could explain the association between BCG immunization and IDDM. However our analysis, data not shown, failed to reveal ethnicity as an explanation for the association. We can not be sure that other confounding variables such as breast feeding or milk consumption could be contributing to the effect. It is reassuring however that the odds ratio in both case control studies were similar and resembled that from the ecological study.

The mechanism by which BCG immunization starting after 1 year of life is associated with IDDM is not known. One possibility is that immunization stimulates the immune system and exacerbates subclinical inflammation in the islet cells. This is particular true of BCG, a live vaccine, since infections are known to break T-cell tolerance through activation of alternative reaction pathways (9). This mechanism may involve release of interferons and other cytokines associated with IDDM (10) (11) as well as up regulating MHC molecules on the islet cells. It is also possible that immunization may release viruses in chronically infected individuals (12) and these viruses may speed the destruction of islet cells. Furthermore BCG has at least one antigen that cross react to islet cells (13) and this antigens may stimulate an autoimmune disease. It is possible that immunization also alters the ratios of Th1/Th2 CD4 lymphocytes (14).

This study demonstrates that certain methods of analysis should be used in order to consistently see an effect of vaccines on IDDM. The time between immunization and the onset of IDDM must be considered. A study should start enrolling children since the time of immunization and follow them for 7 or more years in order to prevent missing an effect of immunization on IDDM. When studying the effect of a particular vaccine on IDDM one must control for changes

in other vaccines since the immunization practices of several vaccines may be changed at similar times. For example, vaccination practices with the BCG and smallpox vaccines were changed at similar times in Sweden (15) and the effect of the smallpox vaccine may have had the opposite effects as the BCG vaccine.

Proper immunization records need to be kept as discrepancies may persists as to what vaccines the study group received since published records may differ on the extent of immunization (15). If immunization approaches 100% in the total population then the immunization rate in diabetes and cases will approach 100% and cases control study will show little or no effect of vaccination on IDDM. In certain times after the introduction of a new vaccine children born after a certain year may all have been vaccinated and children born before a certain year are all unvaccinated. In this situation the immunization rate may appear substantially below 100% making it appear as if a case control study is feasible. However, if all the controls are strictly matched by date of birth with the cases then the immunizations in the cases and controls will be the same. This phenomenon may explain results of a Swedish case control study (16,17). A similar phenomenon can occur when controls and cases are closely matched by area of residency or school distinct and vaccination differs by one of these geographic boundaries.

Differences between studies may be due to real differences in vaccines and formulations of vaccines. Live viral vaccines like the live polio vaccine probably alter the immune system quite differently than the BCG vaccine or killed polio vaccines. Killed whole cell bacterial vaccines with aluminum adjuvants probably alter the immune system differently than killed whole cell vaccines lacking aluminum adjuvants. The plague vaccine which lacks an aluminum adjuvant had less of an effect on mice than even lower doses of the anthrax vaccine which contained aluminum adjuvants (1). The old Swedish whole cell pertussis vaccine which lacked an aluminum adjuvant may have little effect on IDDM, alternatively DT vaccines with aluminum adjuvants may have an effect on IDDM equal to a DTP vaccine lacking an aluminum adjuvant (18).

The power of studies looking at the effect of vaccines on IDDM is very important when interpreting the results. Immunization rates are high for many vaccines, approaching 100%, so even odds ratios of 1.25 are clinically very important. The effect of these seemingly small odds ratios is even more important when one considers children may receive 10 or more different vaccines before adulthood and these vaccines may have an additive effect on IDDM. For example BCG immunization was associated with an increased risk of IDDM in children under 18, odds ratio of 2 to 3, in this paper. This suggests that in some countries where all children receive BCG starting at school age the incidence of IDDM may be reduced 50% by discontinuing immunization with BCG or giving the vaccine starting at birth.

There are several methods that can be used to increase the power of detecting an effect of immunization on IDDM. One method is to limit the analysis to the first few years after immunization. If the analysis is limited just to children a few years after immunization the odds ratio is likely to be much higher since children are more likely to develop vaccine induced diabetes within four years of immunization as opposed to 15 years after immunization. The statistical tools one use is also very important. A single tail test will have twice the power of a two tail test. Based on the consistent results in animals and humans it is reasonable to use a single

tail analysis when testing the hypothesis that immunization at birth is associated with a decreased risk of IDDM or immunization starting after 2 months is associated with an increased risk of IDDM. It is also reasonable to use parametric procedures such as a normal approximation of Poisson distribution as opposed to relying on chi square and other non-parametric procedures. The former methods are more powerful because they are based on the assumption that there is an underlying linear relationship regarding the development of IDDM over time. Data from Sweden (19) indicates that the cumulative incidence of IDDM rises in an approximately linear pattern and thus supports the use of parametric procedures.

These studies support the ecological data that BCG immunization starting after 1 year is associated with an increased risk of IDDM in humans. Additional studies are underway to confirm this association. Preliminary data from Denmark, which routinely administered the BCG vaccine to children at age 7 prior to discontinuation of the vaccine around 1990, showed the incidence of IDDM decline in children under 15 (20). The average incidence was 19.2 cases/100,000 in 1989-1990 and declined to 15.8 cases/100,000 in 1992-1993, a drop in cumulative risk of 50 cases/100,000 by age 15. Preliminary data from the UK further revealed that the health regions encompassing the cities of York and Hull, which discontinued BCG immunization at school age, had a lower incidence of IDDM then the surrounding areas which continue to give the BCG vaccine (21).

Currently in Western Europe some countries give BCG starting at birth, some starting at school age and some do not give the vaccine at all. Due to the acceptance of different immunization practices with BCG in industrialized nations it is feasible to do large clinical trials to study the effect of BCG immunization practices on the incidence of IDDM. Given the odds ratio of approximately 2 and the high immunization rates a clinical trial should be seriously considered. Results from the studies may result in changes in BCG immunization practices, a substantial reduction in IDDM, and provide insight into evaluating the safety of other vaccines.

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Table 1: Series A, Children Age 7-18

Age 7-18	Diabetics	Controls
No BCG	72	2256
BCG first year of life	15	497
BCG starting at age 1-4	2	108
BCG starting at age 5-6	1	11
BCG starting at 7-18	3	4
BCG age unknown	0	27
Total	93	2903

Table 2: Series B, Children Age 0-18

	Diabetics	Controls
No BCG	205	366
BCG year 1 of life	30	53
BCG starting after age 1	14	12
Total	249	431

Table 3: Calculations

Series A, Children Age 7-18

	Diabetes	Control	ODDs ratio:	95% Confidence	Chi square	Fisher, 1 tail
Analysis #1						
BCG exposure starting after age 7	3	31	3.09	(0.93, 10.29	p=0.053	p=0.087
Remaining children	90	2872				

	Diabetes	Control	ODDs ratio:	95% Confidence	Chi square	Fisher, 1 tail
Analysis #2						
BCG exposure starting after age 5	4	42				
Remaining children	89	2861	3.06	(1.07, 8.72)	p=0.027	p=0.053

Series B, Children Age 0-18

	Diabetes	Control	ODDs ratio:	95% Confidence	Chi square	Fisher, 1 tail
BCG exposure starting after age 1	14	12				
Remaining children	235	419	2.08	(0.95, 4.57)	p=0.063	p=0.051

Stratified Statistical Analysis Combining Series A (#1) and Series B

ODDs ratio:	95% Confidence	Chi square
2.3	(1.13, 4.95)	p=0.019